

# Cell Differentiation Is a Primary Growth Process in Developing Limbs of *Artemia*

JOHN A. FREEMAN\*

*Department of Biological Sciences, University of South Alabama, Mobile, Alabama 36688*

**Abstract.** The limb of the brine shrimp *Artemia* develops over a four-instar period when the protopod, endite, exopod, endopod, and epipod are defined and cell differentiation (change in cell shape) occurs. To understand the importance of cell differentiation in limb growth, development of the epidermis was studied in the first thoracopod of instar V–VIII larvae. Each region was established by instar V, and the larval epidermal cells developed into general epidermal (GEC), tendinal, setal, or transport cells by instar VI. Basal extensions of the GECs formed pillar structures. The epidermal cells decreased in height from 10 to 4  $\mu\text{m}$  by instar VI. Increase in length and width resulted from both cell replication and expansion of the apical cell surface in differentiating cells. Growth occurred mainly by cell replication in instar V, whereas expansion of the cell surface in GEC and setae was the major growth process in instar VII. Increase in apical cell surface area occurred primarily by change in cell shape from columnar to squamous during instar V and by increase in total cell surface in subsequent instars. The results demonstrated that cell differentiation is a significant component of growth during limb development.

## Introduction

Crustacean larvae grow by increase in tissue mass during the molt cycle and the periodic shedding of the restrictive exoskeleton. Nutrients stored during the intermolt period support cell growth during the premolt period (Hartnoll, 1982; Freeman, 1991). The increase in dimension and change in shape is realized after ecdysis when the newly formed cuticle unfolds and stretches (Cheng and Chang, 1993, 1994). Moreover, regions of the integument may grow at different rates (Blake, 1979; Hartnoll, 1982).

In developing crustaceans, new structures formed during one instar become evident after the molt to the next instar (Schrehardt, 1987). The apical surface area increases when columnar larval cells flatten, becoming squamous general epidermal cells (GECs). Later these cells continue to grow by increasing the total surface area. Both processes lead to a larger cuticle-forming surface (Freeman 1989, 1993, 1995). As the GECs differentiate, they may produce basal pillar structures that maintain the paddle shape of the limb (Benesch, 1969; Taylor and Taylor, 1992). Other specialized cells include tendinal cells that attach muscles to the cuticle (Freeman *et al.*, 1995), cells that form the setal shaft (Tchernigovtzeff, 1976; MacRae and Freeman, 1995), and cells that are modified for respiratory and osmoregulatory functions in the epipod (Copeland, 1967).

Many of these cell types change shape as they differentiate, and this change may contribute to growth of the appendage by increasing the area of the apical surface. To understand the role of cell differentiation in limb growth and development, cell replication, cell shape change, and apical surface expansion were examined in the first thoracic limb of *Artemia* during instars V–VIII. In addition, the amount of new plasma membrane needed to support growth by cell replication and cell enlargement was estimated for two growth periods. The results demonstrate that change in cell shape during differentiation leads to growth of the integument and formation of the limbs.

## Materials and Methods

Brine shrimp were reared in filtered seawater, salinity 30 ppt, at 23 °C. Limb structures were measured in live larvae or in specimens fixed in 3:1 ethanol-acetic acid and stained for nuclei with Hoechst 33258 (Sigma Chemical, St. Louis, MO) or for plasma membranes with  $\text{C}_{10}$ -Bodipy<sub>500/515</sub>- $\text{C}_3$  (Molecular Probes, Eugene, OR) (Freeman, 1995; Macho *et al.*, 1996). Linear measurements were made using an ocular

micrometer or through an imaging system calibrated using a slide micrometer. Images were acquired with Inspector software ver. 2.1 (Matrox Electronic Systems, Quebec, Canada).

For determinations of length (proximal-distal axis) and width (medial-lateral axis), the region was measured in the widest part of each axis (see Fig. 1C). Although this method may lead to a slight overestimate due to the shape of some of the lobes, it does compensate for any underestimation due to the convexity of the curved surface. The number of cells was determined by counting the nuclei in the region. The mean apical surface area (cuticle-secreting) per cell was determined by dividing the area of the region by the number of cells. The cellular data were determined for the anterior face of the limb only. Cell height was determined by taking the mean of five measurements in live larvae.

Morphometric determinations of cell height and mean cell surface area formed the basis for computations of membrane biosynthesis. To determine total surface area (apical, lateral, and basal) for each cell, the cell was considered to be a cylinder. Total surface area for the region was obtained by multiplying the value per cell by the number of cells in that region. The values in Table 1 indicate the differences between instars (total surface area for instar  $n + 1$  minus the total surface area for instar  $n$ ). For mitosis, the new membrane for cytokinesis was assumed to bisect the cell. For surface area determinations on setae, the height and base were used to compute the surface area of a cone. The diameter of the setal base was measured directly at high magnification, and the plasma membrane was evident at the tip of the setae. The amount of new surface membrane produced for mitosis or cell expansion is indicated as differences between instars (Table 1). Cells that increased their apical surface by becoming squamous (SC in Table 1) were considered to have changed shape without an increase in total cell surface area.

## Results

### *Limb morphology*

The adult limb is shown in Figure 1. The protopod has medial (endites, endopod) and lateral (exopod, epipod) lobes. Setae and muscles can be seen in Figure 1A, and differences in cell (nuclei) density are shown in Figure 1B. The adult limb reached 1100  $\mu\text{m}$ , and the length and width of each region (Fig. 1C) are indicated in Figure 1D. In instars V through VIII, the limb grew primarily in length, and by instar VIII the limb dimensions were 25% that of the adult (Fig. 1E).

### *Growth and regional differentiation*

At the beginning of instar IV, the limb is a phyllopod with 150 cells on the anterior surface (Fig. 2A). After apolysis,

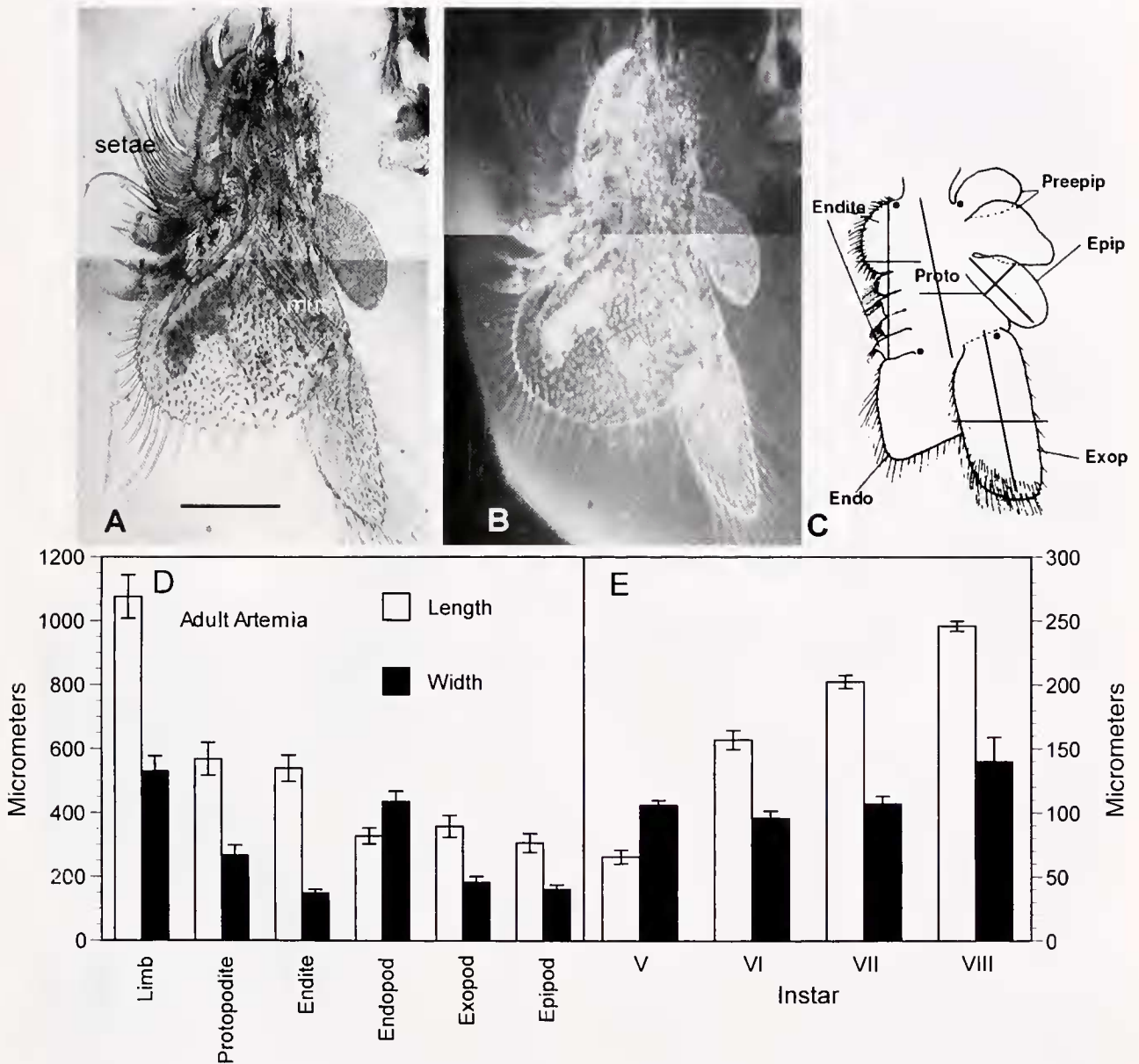
invaginations separating prospective lobes were evident at the distal edges. Cell division in the proximal-distal axis of each lobe (Fig. 2B) doubled the number of cells in the lobe by late instar IV (Fig. 2C). At the molt to instar V, the endites and endopod became evident on the medial edge, and the exopod and epipod formed on the lateral edge of the protopodite (Fig. 2D, E). Each of these regions was composed of densely packed larval cells 10–12  $\mu\text{m}$  in height (Fig. 2E).

Differentiated cell types began to develop in instars V and VI. Larval cells became squamous general epidermal cells (GECs) in the protopod in instar V as their height decreased from 10  $\mu\text{m}$  to 3–5  $\mu\text{m}$  (Fig. 2F, G). The tendinal cells, setal cells, and pillar structures were clearly defined early in instar VI (Fig. 2G, H, I). The general form of the limb with all cell types in each region was attained by instar VIII (Fig. 2J).

As the phyllopod developed, growth of the limb resulted in a marked increase in length and width (Fig. 1E). However, due to presence of numerous pillar structures, each an extension of the basal surface on the anterior surface that attaches to a like process extending from the GECs on the posterior surface, the maximum depth of the limb remained at 60–80  $\mu\text{m}$  through instar VIII (Fig. 2I). Based on a diameter of 2  $\mu\text{m}$  and a length of 20  $\mu\text{m}$ , each pillar extension had a surface area of 63  $\mu\text{m}^2$  per cell. Although the absolute number of pillars was not determined for each lobe, the number of pillars in some regions equaled the number of GECs, suggesting that, in these regions, each GEC produced a pillar extension.

The protopod grew in length at each instar. The region was wider than long in instar V but doubled in length by instar VI (Fig. 3A, E). By instar VIII the region had grown 3-fold in length and 30% in width (Fig. 3E). With the exception of a few tendinal cells, GECs populated the protopod surface. Initially, these cells were arranged in four medial-lateral rows (Fig. 3A). There were about 24 cells in the protopod of instar IV, 100 cells in late instar VI, and 160 cells in instar VIII (Fig. 3F). The height of the cells declined from 10 to 4  $\mu\text{m}$  by instar VI, and the apical surface grew during premolt; these changes resulted in undulations in the epidermal surface in late premolt (Fig. 3B, C). The mean apical surface area per cell decreased slightly from instar V to VI, when over half the cells replicated, but it then increased continuously for the next two instars (Fig. 3F). By the adult stage, the mean surface area increased to 597  $\mu\text{m}^2$  (Fig. 3D). Tendinal cells were evident by instar VI. The results indicate that growth of the protopod was a result of cell replication, shape change, and apical surface expansion.

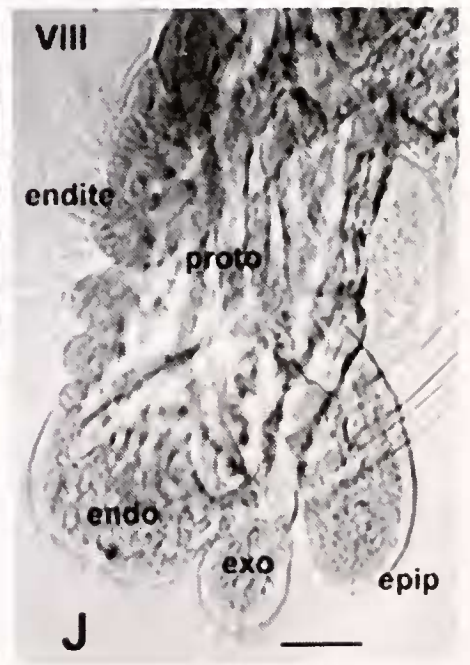
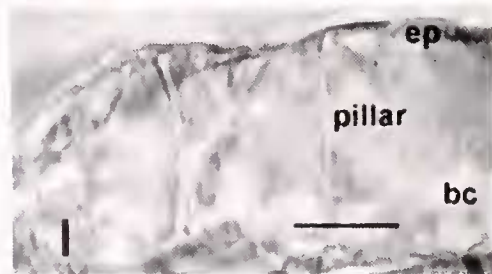
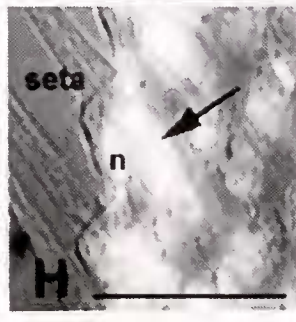
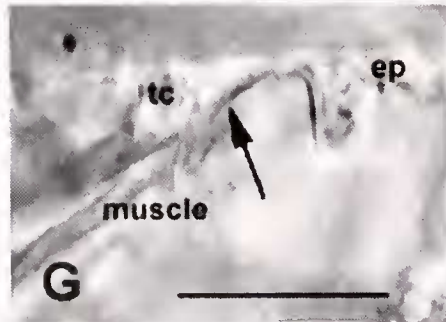
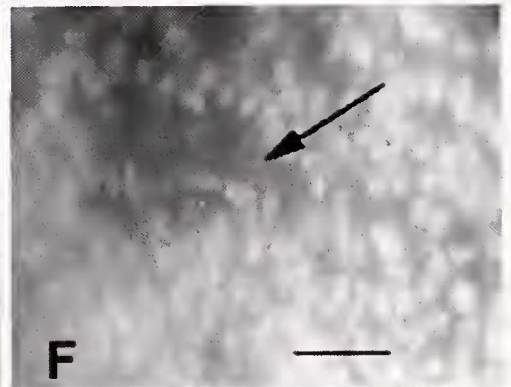
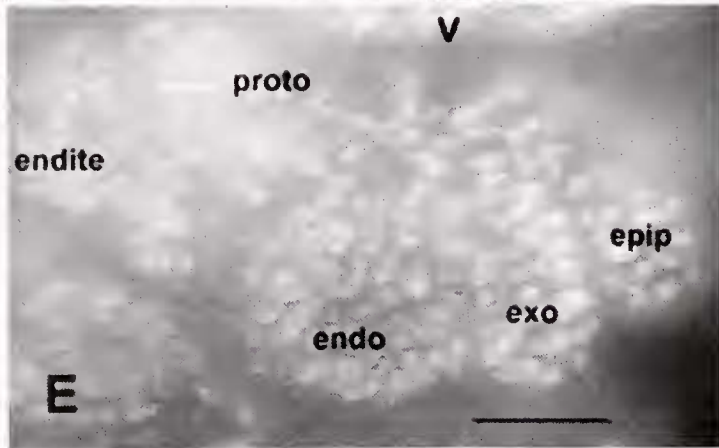
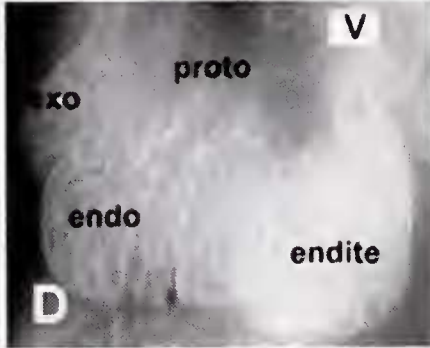
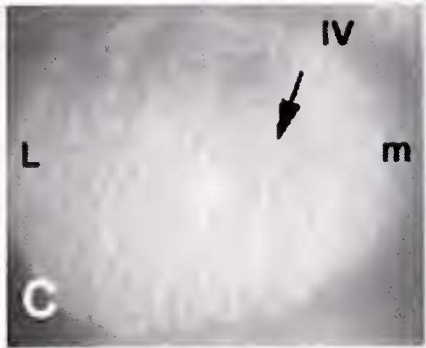
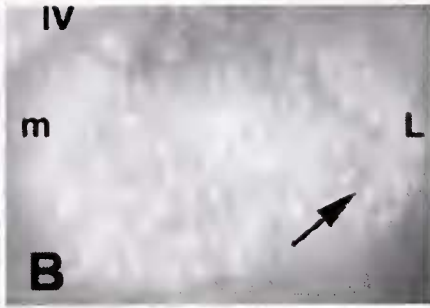
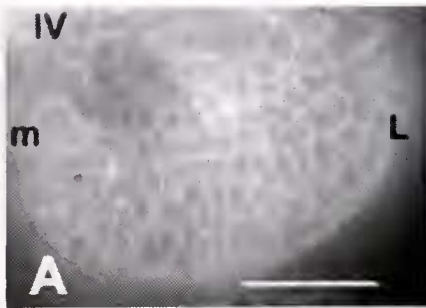
The endites include four setated lobes on the medial edge of the limb bud (Fig. 1). Setae were evident by instar V and increased in number and length through instar VIII (Fig. 4A, F). Setal cells developed in distal regions associated with high cell density, whereas GECs formed in more proximal

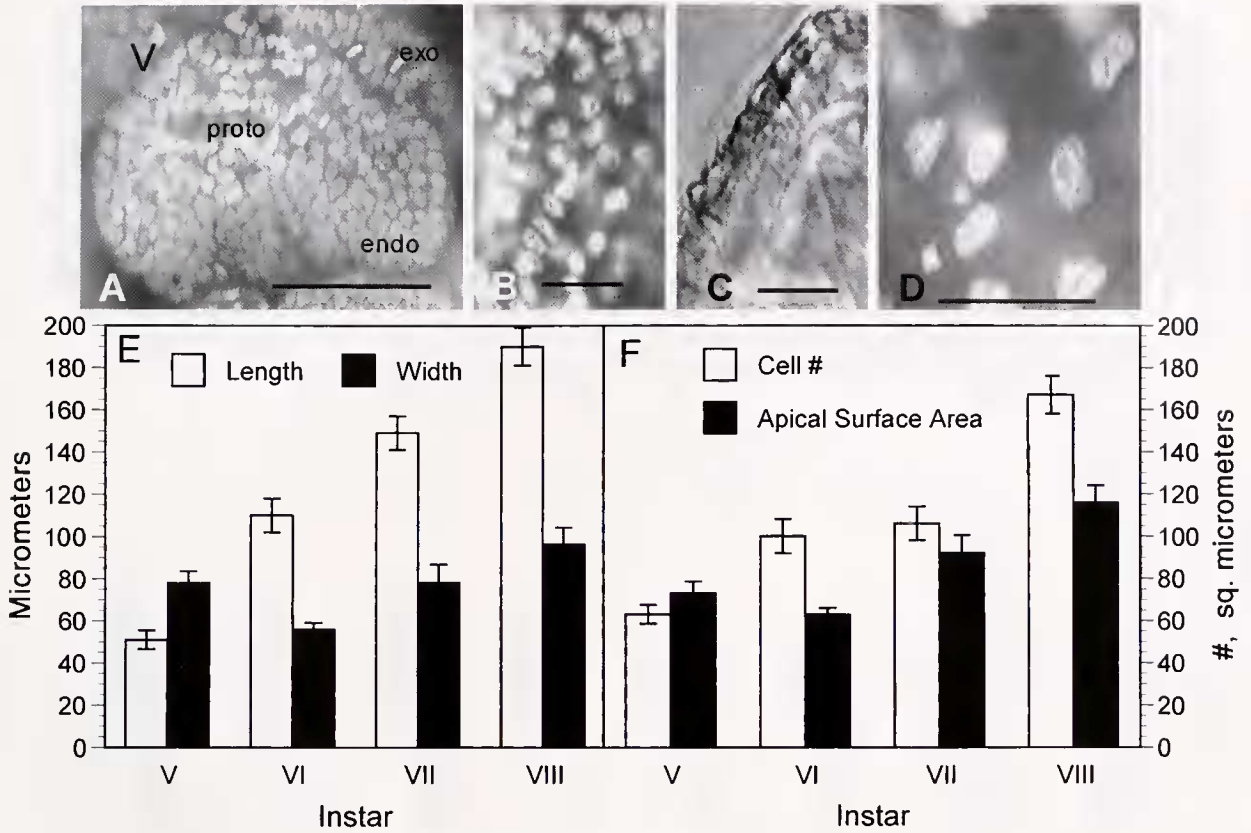


**Figure 1.** The adult thoracopod of segment I as seen in light (A) and fluorescence (B, for nuclei) images. Setae and muscles (mus) are indicated in A. The different regions are indicated in the diagrammatic view (C, adapted from McLaughlin, 1980, with permission from W.H. Freeman and Company), and the corners of the protopod are delineated by black dots. The axes used for length and width determinations are indicated. For clarity, only the bases of the setae are shown. Medial is to the left in each view. Abbreviations in C: proto, protopod; endo, endopod; exop, exopod; epip, epipod; prepip, prepipod. Bar in A (=B) represents 200  $\mu\text{m}$ . (D) Length and width of the adult limb and the lobes. Each bar in D indicates the mean  $\pm$  1 SD of 8–16 larvae. (E) Dimensions of the first thoracopod in instars V–VIII. Each bar in E indicates the mean  $\pm$  1 SD of 10–18 measurements.

regions adjacent to the protopod (Fig. 4A, B, C). The region increased in length, with a slight increase in width through instars V–VIII (Fig. 4E). The medial endite region had grown to 500  $\mu\text{m}$  in length and was 140  $\mu\text{m}$  in width in adults (Fig. 1D). Most of this growth was due to cell replication (Fig. 4B, F). The apical surface area increased

slowly during instars V–VIII and then increased 3-fold by the adult (Fig. 4B, D, F). Newly formed setae had a mean length of 25  $\mu\text{m}$ , an increase in apical surface area of 100  $\mu\text{m}^2$  (Fig. 4F). By instar VIII the number of setae increased to 43 and the length reached 87  $\mu\text{m}$ , attaining a surface area of 547  $\mu\text{m}^2$  per seta. In adults, the number of setae had





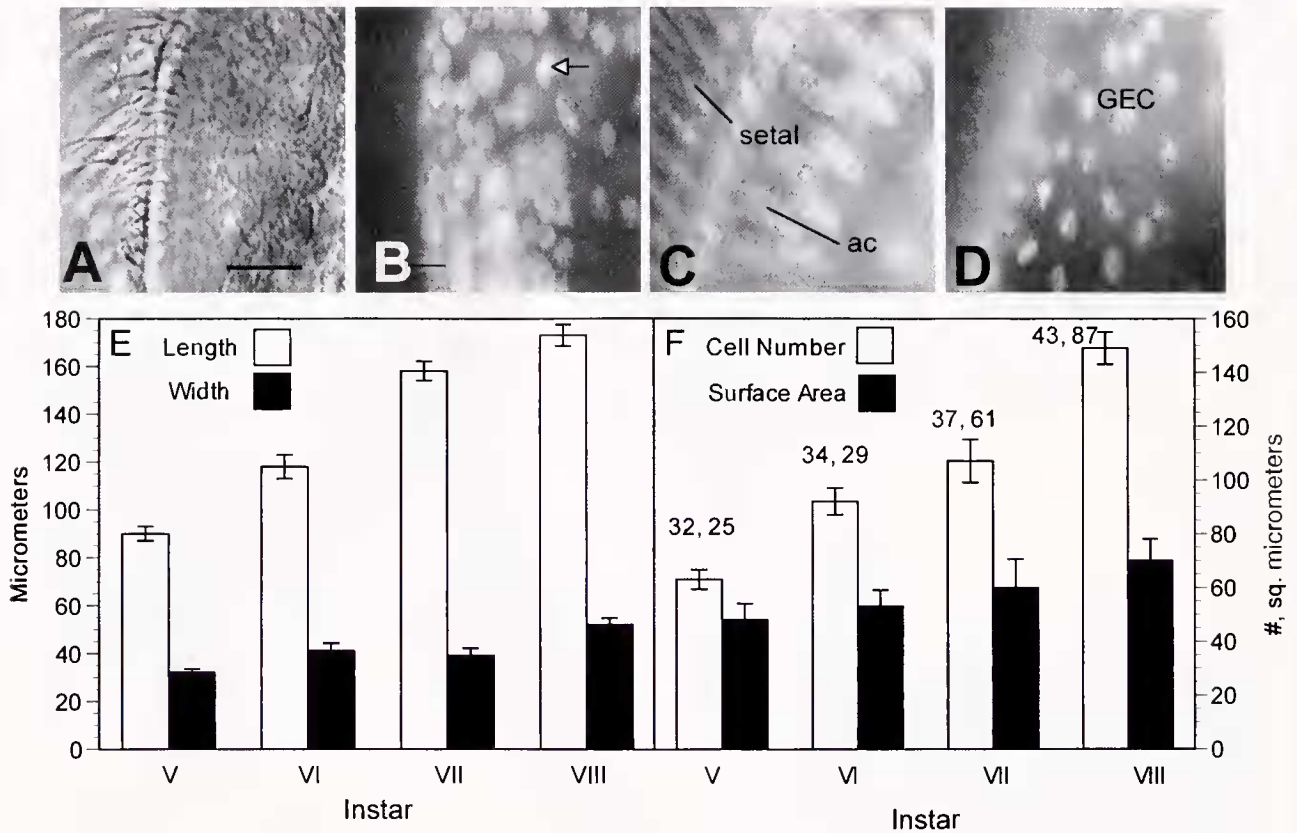
**Figure 3.** Development of the protopod. (A) Cell division and increased apical surface area in instar V. For comparison, the endopod (endo) and the exopod (exo) are labeled. (B) Instar VI stained for nuclei, showing increased surface area (decreased nuclear density) in GECs to the right in the image. (C) Transverse view of epidermal cells, showing undulations of the apical surface late in instar VI. (D) Fluorescent images of GECs in adult limb, showing increased apical surface area. The small nuclei are in blood cells below the plane of the epidermis. Bars in A = 40  $\mu\text{m}$ ; B = 30  $\mu\text{m}$ ; C = 10  $\mu\text{m}$ ; D = 20  $\mu\text{m}$ . (E) Dimensions of the protopodite in instars V–VIII. Each bar indicates the mean  $\pm$  1 SD of 7–15 larvae. (F) Cell number and mean apical surface area during instars V–VIII. Each bar represents the mean  $\pm$  1 SD of 5–7 larvae.

almost doubled, reaching 71, and the length increased to 250  $\mu\text{m}$ . The endites grew by cell replication, increase in surface area, and setal enlargement.

The endopod was a broad lobe with numerous pillars (Figs. 2E, J; 5A, B). It grew in width more than in length,

becoming a thin paddle 98  $\mu\text{m}$  wide by instar VIII (Fig. 5C). It reached 317  $\mu\text{m}$  long and 420  $\mu\text{m}$  wide by the adult (Figs. 1D, 5B). While the number of GECs increased from 34 in instar VI to 50 in instar VIII, reaching 90 cells per surface in the adult, the surface area increased from 42  $\mu\text{m}^2$

**Figure 2.** Development of limb regions in instars IV–VIII. A–C. Instar IV (m, medial; L, lateral). (A) Cell borders early in the instar. (B) Fluorescent image of late instar IV showing mitoses (arrow). (C) Late instar IV limb showing invaginations (arrow) separating lobes late in the instar. (D) Postmolt instar V stained for plasma membrane, showing major regions of the limb. The epipodite is out of focus behind the exopodite. (E) Instar V stained for nuclei. Mitotic figures are evident in the protopod, exopod, and epipod. (F) Increase in apical surface area (lower density of nuclei, arrow) is seen in the protopod in instar V. (G) The tendinal cell (tc) in instar VI connects the muscle to the cuticle secreted by the epidermis (ep). The arrow indicates the region of the cell enriched for microtubules and actin (Freeman *et al.*, 1995). (H) Setae are apical extensions of the epidermal cells ( $n$  = nucleus of setal cell) and have polyploid accessory cells (arrow, nucleus of accessory cell) below the epithelium that aid in the expansion of the seta as it grows during late premolt. (I) Pillar structures extend basally from epidermal cells (ep) on opposite faces of the limb. A blood cell (bc) is present in the spaces between pillar structures. (J) Instar VIII limb. Abbreviations as in Figure 1. Bars in A (=B–D) = 25  $\mu\text{m}$ ; E–I = 20  $\mu\text{m}$ ; J = 35  $\mu\text{m}$ .



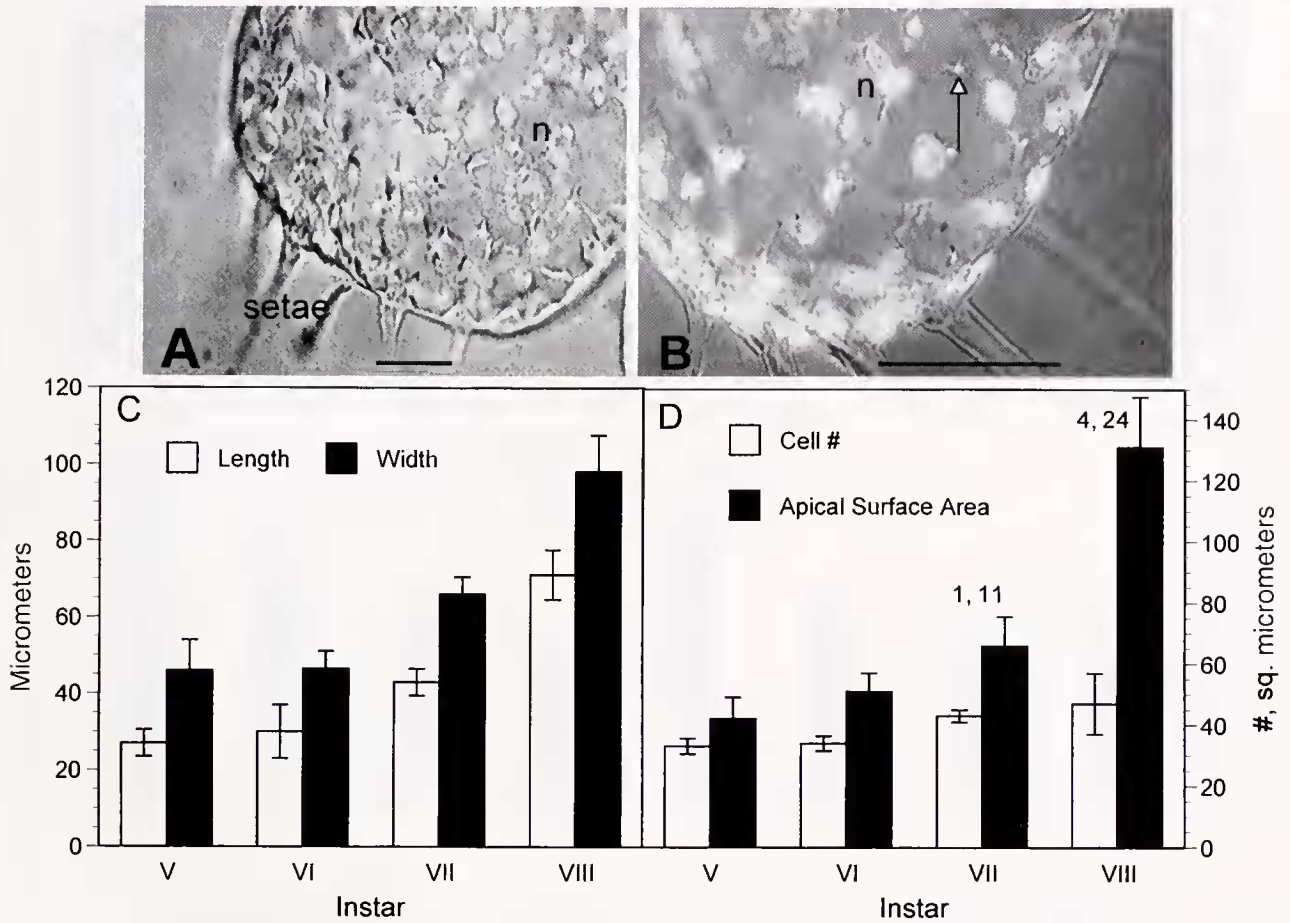
**Figure 4.** Development of the endites. (A, B) Light and fluorescent (for nuclei) images, respectively, of instar VI endite. The setal cells are to the left and the GECs are to the right in each image. Line in B indicates the edge of the region where setae are forming. The arrow indicates a mitotic figure. (C, D) Same adult endite viewed at different focal planes, showing setal cell nuclei (setal), accessory cells (ac), and GEC nuclei. Bar in A (=B–D) = 20  $\mu\text{m}$ . (E) Dimensions of the endite during instars V–VIII. Each bar indicates the mean  $\pm$  1 SD of 6–10 larvae. (F) Cell number and mean apical surface area in endite epidermal cells during instars V–VIII. Numbers above bars indicate number of setae and mean length ( $\mu\text{m}$ ) of the setae for that instar. Each bar represents the mean  $\pm$  1 SD of 4–6 larvae.

in instar V to 131  $\mu\text{m}^2$  in instar VIII and 542  $\mu\text{m}^2$  by the adult (Fig. 5B, D). Setae began to form during instar VII and increased from four 24- $\mu\text{m}$ -long setae in instar VIII to 22 setae with a mean length of 172  $\mu\text{m}$  in the adult. Tendinal cells formed at least seven muscle attachments during instar V. Apical surface expansion was the primary means of growth in this region.

The exopod was first evident as a small bud of 15 cells per surface in instar V (Figs. 2E, 6A). It continued to grow more in length than in width, becoming a thin blade by instar VIII (Fig. 6B, C). It grew from 20  $\mu\text{m}$  in instar V to 51  $\mu\text{m}$  at instar VIII and to 300  $\mu\text{m}$  in the adult (Figs. 1D; 6E). Cell replication increased the cell number from 18 in instar V to 30 by instar VIII (Fig. 6F). Ninety GECs were present in the adult. The apical surface area doubled by instar VIII and reached 212  $\mu\text{m}^2$  by the adult (Fig. 6D, F). The GECs became arranged in proximal-distal rows that alternated with setal cells (Fig. 6D). There were three or four small setae in instar VIII. In the adult, the number increased to 21 setae, and the mean length reached 363  $\mu\text{m}$ .

Two muscles attached to tendinal cells. This region grew primarily by expansion of the cell surface.

The epipod was first evident as a small lobe in instar V (Fig. 2E). As the GECs differentiated, the limb grew through cell replication and apical surface expansion to form a flattened sac (Fig. 7A, B). The lobe grew to 96  $\mu\text{m}$  in length by instar VIII (Fig. 7D), reaching 300  $\mu\text{m}$  in the adult (Fig. 1D). The number of cells increased slightly in instars V–VIII and increased to 50 per surface in the adult (Fig. 7E). The height of the cells decreased from 8–10  $\mu\text{m}$  in instar V to 4  $\mu\text{m}$  in instar VI. There was a gradual change in apical cell surface from 44  $\mu\text{m}^2$  in instar V to 162  $\mu\text{m}^2$  in instar VIII, eventually reaching 1020  $\mu\text{m}^2$  in the adult (Fig. 7C, E). The cells appeared to be arranged in two populations, one forming the narrow edge of the lobe and the other forming the flat surface supported by pillar structures (Fig. 7A, C). There were about 60 pillars in the adult. No muscles attached to this lobe and no setae formed. Most of the growth was a result of expansion of the apical surface.



**Figure 5.** Development of the endopod. (A) Endopod in instar VIII, showing newly formed setae and GEC nuclei (n). (B) Light/fluorescent image of adult endopod, showing nucleus of GECs (n) and pillar structure (arrow). Bars in A and B = 20 and 50  $\mu\text{m}$ , respectively. (C) Dimensions of the endopod during instars V–VIII. Each bar indicates the mean  $\pm$  1 SD of 8–12 larvae. (D) Cell number and mean apical surface area for instars V–VIII. Numbers above bars indicate the mean number and length of new setae. Each bar represents the mean  $\pm$  1 SD of 6–12 larvae.

#### Production of new cell surface

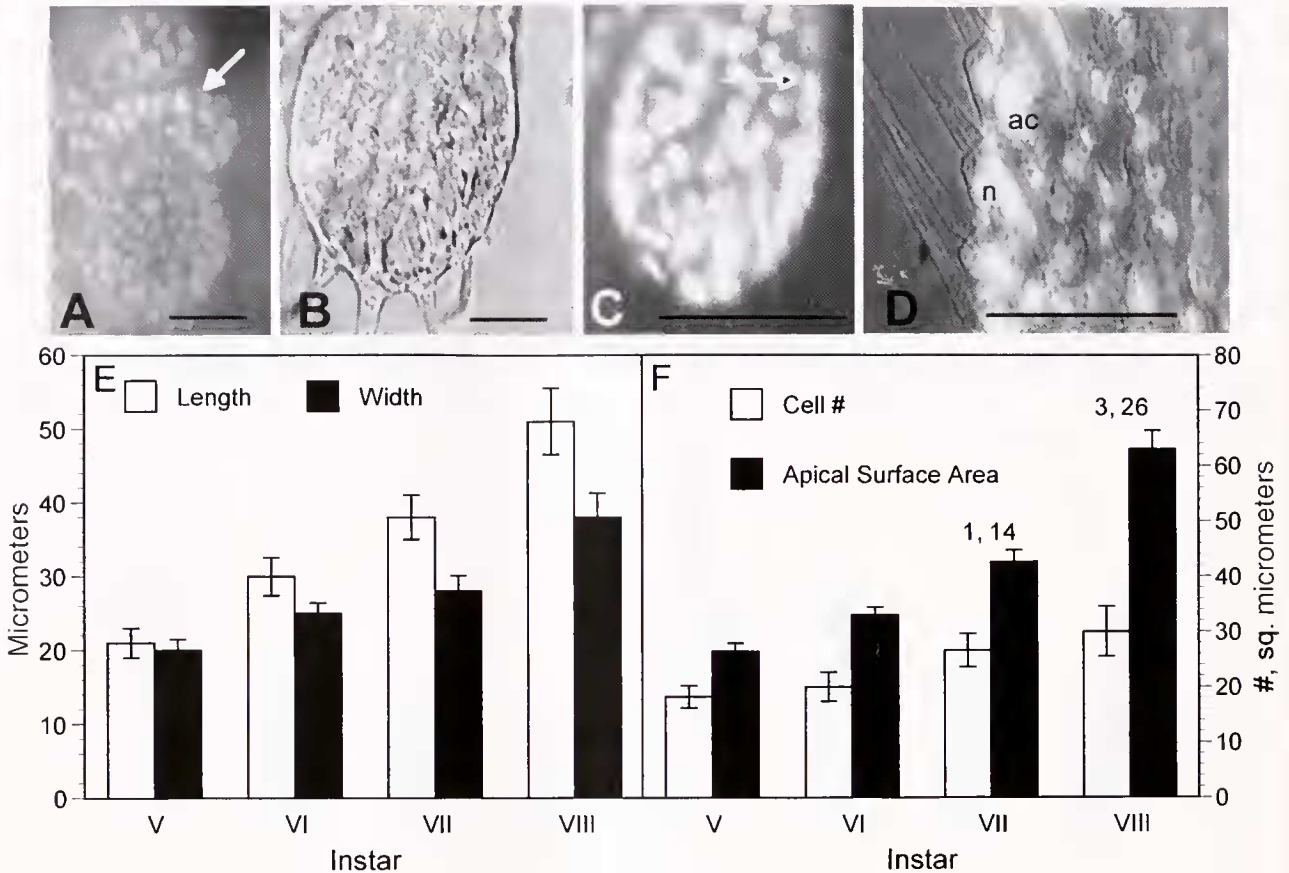
The morphometric findings demonstrated that development of the limb involved both cell replication and expansion of the apical surface. Cytokinesis, expansion of the apical membrane surface in GECs, and formation of setae require production of new plasma membrane. To assess the importance of new membrane production associated with the growth and developmental processes, the morphometric data (Figs. 3–7) were used to estimate the amount of increased cell surface area needed to support cytokinesis and increased apical surface area in each region of the limb (Table 1).

Growth from instar V to VI (total surface area of instar VI – total surface area of instar V) involved synthesis of new membrane for cytokinesis. Elongation of the setae occurred in the endites. Cells in the endites also produced new membrane for the cell surface as the region elongated

in the proximal-distal axis while cells in the protopod, endopod, and epipod changed shape from cuboidal to squamous, presumably increasing the apical surface area without significant production of new plasma membrane (SC, Table 1). In instar VII, all regions demonstrated production of membrane for cytokinesis, and the level was 33% greater than in instar V. The increase in total cell surface, however, was much greater than that observed in instar V and 4 times the area formed by mitosis. The setae continued to grow in the endites, and new setae developed in the endopod and exopod. Overall, cell surface expansion in instar VII was over 6 times that found for instar V.

#### Discussion

The crustacean integument grows during the molt cycle, resulting in an expanded cuticle at the next ecdysis. General patterns included cell replication early in the molt cycle



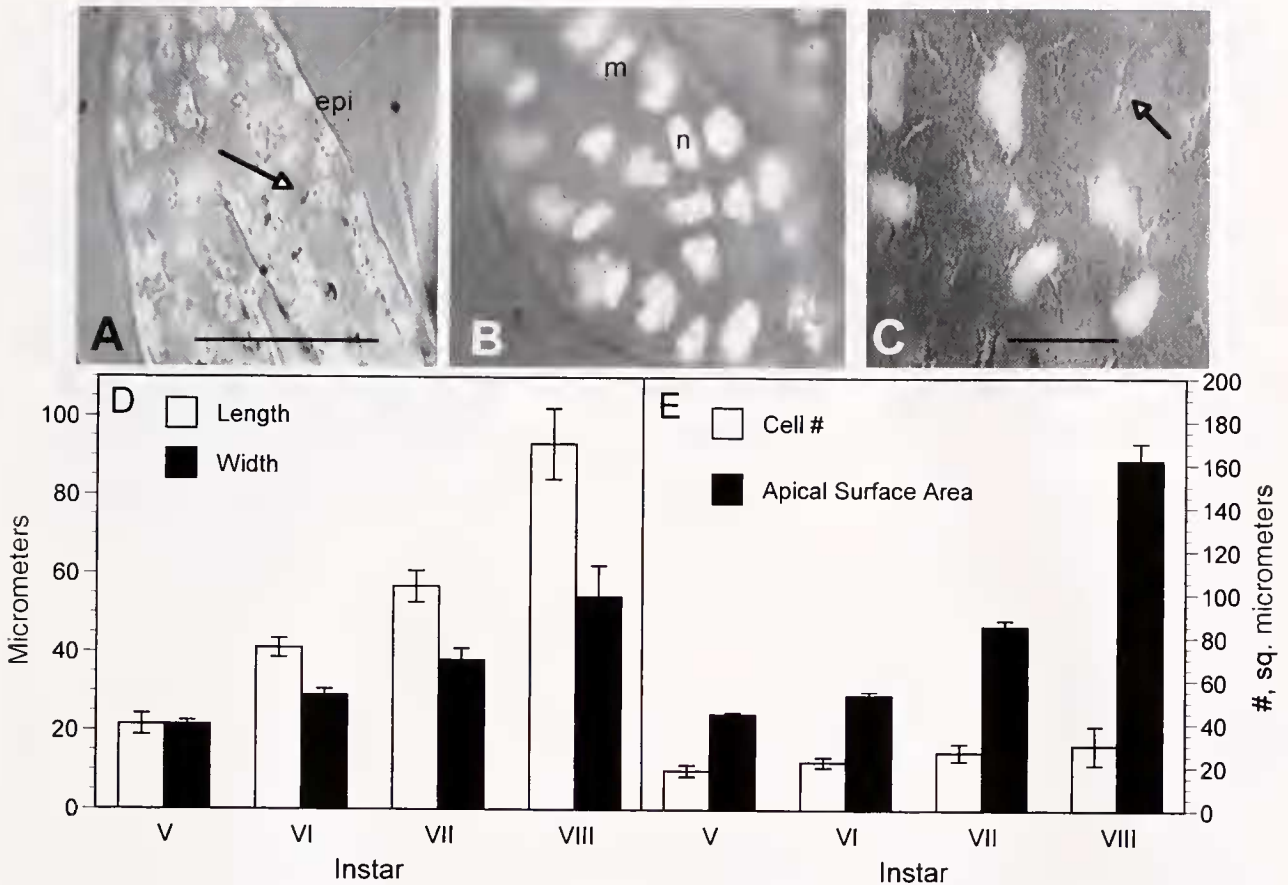
**Figure 6.** Development of the exopod. (A) Exopod in instar V limb. (B, C) Light and fluorescence images, respectively, of exopod in instar VIII, showing new setae and nuclei of GECs. Arrows in A and C indicate mitotic figures. (D) Exopod of adult, showing the setal region at the edge of the lobe. Nucleus (n) of a setal cell and an accessory cell (ac) are shown. Bars in A = 20  $\mu\text{m}$ , B = 15  $\mu\text{m}$ , C = 40  $\mu\text{m}$ , D = 50  $\mu\text{m}$ . (E) Dimensions of the exopod during instars V–VIII. Each bar indicates the mean  $\pm$  1 SD of 6–12 larvae. (F) Cell number and mean apical surface area for GECs in instars V–VIII. Numbers above the bars indicate the mean number and length of setae for those instars. Each bar represents the mean  $\pm$  1 SD of 5–12 limbs.

with subsequent growth during late premolt (Freeman, 1991), or replication during mid-premolt that resulted in an undulated apical surface (Cheng and Chang, 1994). The results reported here reveal another process, expansion of the apical surface as the cell differentiates to a general epidermal cell (GEC) or a setal cell. These changes complement cell replication and are essential to growth and development of form in larval crustaceans. Of the four cell types studied, the GEC, setal, and transport cells contributed to growth as they differentiated. Another morphogenic force, evident in the transition from instar V to instar VI, involved a change in cell shape (SC in Table 1) from columnar to squamous, with minimum formation of new plasma membrane. It is possible that the elongation of the limb in the proximal-distal axis involved flattening of some of the GECs and a reorganization of cell neighbors in the plane of the epithelium (Schöck and Perrimon, 2002). The mechanisms by which these changes occur and new mem-

brane is integrated into the plasma membrane remain to be studied.

Each of the regions of the phyllopod grew in a different spatial and temporal pattern. The protopod grew by cell replication and cell shape change early, and differentiation and cell surface expansion of GECs later. The endites grew by replication, apical surface expansion, and development of setae, although the contribution of each process was different in each instar. The endopod demonstrated apical expansion early, followed by growth in all categories in later instars. The exopod and epipod, by contrast, grew by mitosis initially but by cell surface expansion later on. The results demonstrate that apical surface expansion associated with differentiation contributes significantly to growth of the limb. Further, these differences suggest that form in the limb is determined by the patterns of cell replication and cell differentiation in the epidermis. The limb is not the only region to demonstrate both growth processes; similar results





**Figure 7.** Development of the epipod. (A, B) Light and fluorescent images, respectively, of instar VIII epipod, showing epidermis (epi), mitotic figure (m), and GEC nucleus (n). (C) Epipod of adult. Arrows in A and C indicate pillar structures. Bars in A (=B) = 40  $\mu\text{m}$ ; C = 20  $\mu\text{m}$ . (D) Dimensions of the epipod in instars V–VIII. Each bar indicates the mean and  $\pm 1$  SD of 4–15 limbs. (E) Cell number and mean apical surface area for GECs in instars V–VIII. Each bar represents the mean  $\pm 1$  SD of 2–15 limbs.

were found in the lateral surfaces of the first segment (Freeman, 1995).

GECs formed by shape change and increased apical surface area. Some cells also expanded the basal surface, forming pillar structures connecting the anterior and posterior surfaces of the limb. A few pillars were observed in instar IV. As each region developed, new pillar processes formed, maintaining the paddle-like structure of the appendage as it grew and as it changed shape in the transition from instar V to VI. Formation of a pillar would be one of the first stages of differentiation of the GECs in all the regions of the limb and probably requires a concentration of microtubules and microfilaments in the basal region (MacRae *et al.*, 1991; Taylor and Taylor, 1992; MacRae and Freeman, 1995; Cieluch *et al.*, 2004). Although tendinal cells did not markedly increase the apical surface, their formation also involves reorganization of the basal membrane and cytoplasm as the cells form tonofibrillae and muscle attachments (Freeman *et al.*, 1995).

The larval epidermal cell is polarized, producing the

cuticle at the apical surface, a region enriched with microtubules and microfilaments (MacRae *et al.*, 1991). As the apical surface of the cell expands by insertion of new membrane, the cytoskeleton may be active in supporting the expanded cell borders, the enlarged apical plasma membrane, and the biosynthesis of the new cuticle.

Analysis of changes in surface area during cell differentiation and growth demonstrated the remarkable amount of plasma membrane biosynthesis during cytokinesis and differentiation. When a molt cycle duration of 1 to 2 days is considered, it becomes clear that a large proportion of the nutrients in the diet must be used to produce the protein and phospholipids for new membrane. About 50% of plasma membrane is lipid, and 10%–45% of the essential fatty acids acquired in the diet are incorporated in polar lipid fractions (Navarro *et al.*, 1991, 1992). The importance of these compounds has been demonstrated in studies showing that the level of polar lipids was enhanced in larvae fed diets enriched with polyunsaturated fatty acids and that the levels were maintained when the larvae were subsequently starved

Table 1

Production of new plasma membrane ( $\mu\text{m}^2$ ) by epidermal cells in the developing limb of *Artemia* during two larval instars

Region	Instar V to VI			Instar VII to VIII		
	Mitosis	Expansion	Setal area	Mitosis	Expansion	Setal area
Protopod	4,662	SC	NA	5,856	24,519	NA
Endites	3,654	1,080	1,166	5,880	5,181	11,653
Endopod	88	SC	NA	293	9,007	535
Exopod	185	None	NA	222	2,334	402
Epipod	480	SC	NA	250	6,774	NA
Total=	9,069	1,080 (11,315)	1,166	12,501	47,815 (72,906)	12,590

Expansion indicates an increase in apical surface area supported by biosynthesis of new plasma membrane. Setal area was determined as the area of a cone, using setal height and base diameter minus the original apical surface area. SC indicates an increase due to cell shape change without synthesis of additional plasma membrane. Values in parentheses at bottom indicate the grand total for the period of growth. NA indicates that the region lacks setae.

(Coutteau and Mourente, 1997; Sakamoto *et al.*, 1982). Similar results have been obtained with other crustaceans (Kanazawa and Koshio, 1994). Considering the rapid rate of cellular growth observed in this study, the level of essential fatty acids in the diet becomes critical for optimum cell differentiation.

Several studies have demonstrated variation in the number of larval instars in *Artemia*, and some of the observations shown here are slightly different from those seen in previous studies (Schrehardt, 1987). Variation in instar number has also been shown in other groups of crustacean larvae (Williamson, 1982). Although these differences could be ascribed to different strains of cysts, it is equally possible that the feeding regime affects the number of instars. Knowlton (1974) and McConaughy (1985) have suggested that nutrient allocation for general maintenance, molting, growth, and differentiation occurs during each instar and that there may be a hierarchy in the allocation such that cell differentiation does not occur when the levels of essential nutrients (fatty acids) are low. The cells may allocate nutrients to maintenance and molting at the expense of growth and differentiation. Moreover, cells may replicate but not expand the apical surface (*e.g.*, differentiation of

GECs), resulting in less growth for that instar. If so, these differences could account for variation in the development of structures such as setae, differences in dimension of some of the limb structures, or presence of supernumerary instars. This would be particularly evident in species such as *Artemia* that do not have a well-developed hepatopancreas for long-term nutrient storage and that exhibit many instars in which there is a greater chance of experiencing periods when nutrients are scarce.

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